# Pitfalls in diagnosing Gallbladder Carcinoma – A Single Center Histopathology Study

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## ABSTRACT

**Background**

Gallbladder carcinoma is a rare cancer with incidence of less than 2 per 100,000 populations worldwide. It is the fifth most frequent gastrointestinal malignancy. Radiological or gross examination of majority of gallbladder carcinoma detects no mass. This may lead to under or over diagnosis of cases in histological examination.

**Objective**

To identify pathologic features that contribute to the difficulty in diagnosis of gallbladder carcinoma.

**Method**

Between 2018 and 2023, 22 patients with gallbladder carcinoma were identified using the histopathology registry book at the department of pathology. Blocks, slides, reports and history of those cases were retrieved and reviewed. The slides were analyzed by two or more pathologist noting some of the diagnostic difficulties which could have been encountered. The number and percentage with interpretations of the cases were noted.

**Result**

Nine of 22 primary gallbladder carcinoma cases had tumor masses. Nine cases in histological examination provided diagnostic challenges. The major pitfalls encountered while diagnosing gallbladder carcinoma was mistakenly making a diagnosis of carcinoma when only deeply penetrating Rokitansky–aschoff sinuses are present. Similarly, pathologists misdiagnose carcinoma with minimal disease as benign disease, Adenomyosis as Adenocarcinoma. Under sampling of specimen, grossly occult disease, misinterpreting extracellular mucin pools were other potential pitfalls.

**Conclusion**

Deeply penetrating Rokitansky-aschoff sinus or Adenomyosis can be mistakenly diagnosed as gallbladder carcinoma. Careful attention to any evidence of mural thickening and close examination of deeply situated glandular structures were crucial for proper diagnosis of gallbladder carcinoma.

## KEY WORDS

*Adenomysois, Gallbladder carcinoma, Histology, Necrosis, Pitfalls, Rokitansky–Aschoff sinuses*

## INTRODUCTION

Gallbladder Carcinoma (GBC) is a rare cancer with incidence of less than 2 per 100,000 population's worldwide.[1] It is the most common biliary tract malignancy and the fifth most frequent gastrointestinal malignancy.[2]

GBC is highly fatal disease with a 5 year survival rate of less than 10%. It is the sixth most frequent cancer and second most common gastrointestinal tract malignancy in Nepalese women. Sixty percent of GBC arise in the fundus. Increasing age and the female gender are important risk factors. In addition, smoking, obesity, parity, genetics, chronic bacterial infection, low socioeconomic status, dietary habit, and benign neoplasm of the gallbladder are other risk factors of GBC.[3] GBC are associated with gallstones (80%), porcelain gallbladder (10-20%), and abnormal choledochopancreatic duct junction.[4] Histologically, most cases are pancreatobiliary- type adenocarcinomas, showing variable degrees of differentiation.[5]

Radiological or gross examination of majority of GBC detects no mass. They may present with thickening of Gallbladder wall or mucosal ulceration. Further, changes of gallbladder such as Rokitansky-aschoff sinuses (RAS) may mimic well-differentiated GBC.[6] This leads to under and over diagnosis of cases. Such patients when diagnosed later generally have a poor prognosis. In this study, we reviewed our GBC cases over a six year period, noting some of the pitfalls which could have been encountered. These discussions might be helpful for avoiding the misdiagnosis of cases in the future.

## METHODS

This was a hospital based observational, study conducted in department of pathology, Dhulikhel Hospital, Kathmandu University Hospital (DH, KUH) from January 2018 to December 2023. Twenty-two patients with GBC were identified using the Histopathology registry book. There were 4 males and 18 females. DH, KUH is the national hospital which receives cancer patients referred from all districts of the country and provides health care to more than 250,000 people annually. The research protocol was approved by the Institutional Ethical Review Board of the institution (approval no: 226/23). The cases were patients diagnosed with GBC by histological or cytological examination. Slides and blocks of the cases were retrieved from the stored place of the department. The surgically resected specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin staining. Retrospective analysis of the reports showed the patients presenting with the clinical features of upper abdominal pain, jaundice, weight loss, nausea, vomiting, fever, hepatomegaly, upper abdominal mass/tenderness, and gastrointestinal hemorrhage.

Patients with pathological materials (slides/blocks/reports) present in the Hospital with a diagnosis of carcinoma of gallbladder were included in the study. Inadequate amount of specimen in the block and with lost slides were excluded from the study. Since this is retrospective study with blocks and slides already present in Hospital store, no consent was taken from the patients. The slides were analyzed by two or more pathologist who was completely new to the case. Microsoft Excel sheet 16 was used for the analysis of the data. The pitfalls were observed and the number of cases along with the percentage and the types of pitfalls with interpretations were noted.

## RESULTS

The studied samples were the cases with diagnosis of GBC made in the institution. Nine of 22 primary GBC cases had tumor masses. Age range of tumor was 29 to 78 years with mean age of 58.95 ± 10.77 years. The most common tumor sites were in the fundus (with 4 evident tumors) followed by three on body and two on neck of gallbladder. Thirteen cases it was difficult to see the exact site of the tumor. The different types of GBC are mentioned in the table (Table 1). Nine of our cases presented challenges in the histopathological diagnosis (Table 2).

**Table 1. Different types of GBC and the number of cases (n=32)**

| Types of GBC | n (%) |
|---|---|
| well differentiated adenocarcinoma | 24 (75.0) |
| Moderately differentiated adenocarcinoma | 4 (12.5) |
| Areas with dysplasia | 3 (9.3) |
| Adenosquamous carcinoma | 1 (3.1) |

## DISCUSSIONS

Most of the study suggests that the GBC is two to six times more prevalent in women and the incidence peaks in the seventh decade of life. In our study the female to male ratio was 4.6:1 and the mean age of the diagnosis was 58.95 years. The result are similar to the results of various researchers like Mondal et al., Memon et al. and Mishra et al.[3,6-8] The increase incidence in females might be because of the increase in prevalence of calculi in females.

Ghimire et al. reported 10 (1.28%) cases of GBC out of 783 cases of routine cholecystectomy for gallstone in Nepal. The cases were more common in females (1:2.3).[9]

Sixty percent of GBC arise in the fundus. Our study showed 18.1% cases.[4] No distinct location could be noticed among 59.0% cases.[13] This is similar to the study of Mondal et al.[6]

The preoperative diagnosis of gallbladder carcinoma is a very difficult task. This is related to the disease's non-specific presentation and its similarity to benign biliary tract disorders.[7] The diagnosis is commonly made at an advanced stage because of the vague signs and symptoms

**Table 2. Pitfalls while Histopathological diagnosis of GBC**

| Case | Particulars | Clinical presentation | Diagnosis | Pitfalls |
|---|---|---|---|---|
| A | 76/F | Failed ERCP, Contracted gall bladder, Stent and 3 calculi seen. | Adenocarcinoma | Fragmented bits sent, tumor invading lumen, D/D Mets or primary, |
| B | 62/F | - | Adenocarcinoma | Dysplasia in RAS without tumor mass |
| C | 46/F | - | Adenocarcarcinoma | Adenomyosis with tumor |
| D | 45/F | - | Squamous cell carcinoma | Comedonecrosis (necrosis within tumor nests) seen, cribriform pattern of tumor cells seen. MistakingAdenocarcinomawith SCC |
| E | 54/F | - | Adenocarcarcinoma | Extensive Necrosis overlap with tumor |
| F | 58/F | - | Adenocarcarcinoma | Adenocarcinoma in Acute calculus cholecystitis |
| G | 29/M | GB polyp 1 cm with hypothyroidism | Intracholecytic papillary tubular neoplasm | Lamina propria not seen. Pyloric glands lined back to back. Associated invasion not seen. |
| H | 55/F | - | Dysplasia | Polypoidal structure, Focal low grade dysplasia |
| I | 62/F | - | High grade dysplasia | High grade dysplasia. No invasion |

of disease. Further complicating the situation there is a low sensitivity and specificity of Ultrasound and Computerized Tomography scan in achieving preoperative diagnosis of GBC.[3,10-12]

Various studies have illustrated following potential pitfalls in diagnosing GBC histologically.

•Mistakenly making a diagnosis of GBC when only deeply penetrating RAS are present.

•Misdiagnosing well-differentiated carcinoma with minimal disease as benign disease.[5]

•Adenomyomatosis mistaken for deeply invasive carcinoma.[13]

•Confusing necrosis related to cholecystitis with geographic tumor necrosis.

•Under sampling and with grossly occult disease.

•Misinterpreting extracellular mucin pools.[5]

•The small ducts of Luschka may demonstrate reactive atypia that can be mistaken for invasive adenocarcinoma.[14]

•Differentiating between primary tumors of gallbladder and metastasis.[5,15]

One of the major problems faced by students of pathology while diagnosing adenocarcinoma (well differentiated) of the gallbladder is the difficulty in distinguishing the tumor cells from RAS. These cases can be over or under diagnosed. RAS are continuous, has perpendicular orientation to the surface, and typically have undulating, smooth contours. In contrast, adenocarcinoma has small and variably sized glands, is usually densely packed with angulated contours and is arranged haphazardly.[16] In this situation cytologic atypia with features of increased nuclear cytoplasmic ratio, hyperchromatic nuclei, prominent nucleoli, mitosis, areas of necrosis and desmoplasia should be looked for the diagnosis of adenocarcinoma.[5,17]

A case in our series showed small glands embedded in bundles of smooth muscle and even penetrating the submucosa resembling RAS. The patient (Case A) was a 76 years old female who presents with features of choledocholithiasis and with failed ERCP. Her gallbladder was contracted. Stent and three calculi were seen in the patient's gall bladder. Fragmented bits of the gallbladder were sent in the pathology lab. The surface mucosa was inflamed and also has features of atypia, making it difficult to differentiate from reactive atypia and RAS. The lining cells of deeper glands presented cytological and architectural atypia with significant inflammation (Fig. 1). The surface epithelium exhibited multiple areas with high grade dysplasia. The morphological diagnosis at the time was cholecystitis. Fortunately on deeper section the atypia was more pronounced.

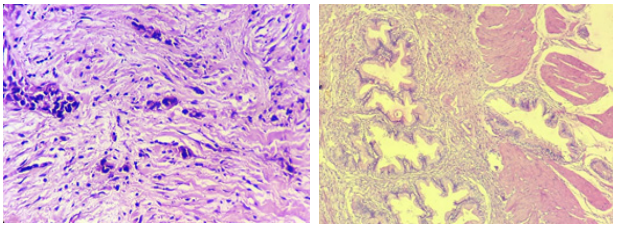

**Figure 1. Left: Atypical cells in gallbladder wall with interspersed inflammatory cells. Right: Normal RAS (H&E, X100)**

Similarly, there can be an urge to diagnose carcinoma when RAS are deeply penetrating and has features of reactivity.

Another potential problem faced by pathologist is carcinoma arising from RAS, without presence of obvious tumor mass.[18] Demonstration of this requires features of carcinoma arising from RAS and also located in the wall or subserosa, with no apparent connection to mucosa. The morphological diagnosis made by the residents in our case (Case B) was invasive moderately differentiated adenocarcinoma, though no mass was identifiable grossly and the wall only focally thickened to 5 mm. Subsequent review of the deeper section showed surface dysplasia, with dysplastic epithelium within RAS penetrating slightly beyond the muscular layer (Fig. 2). The gradual transition between adenocarcinoma cells and RAS with dysplasia was

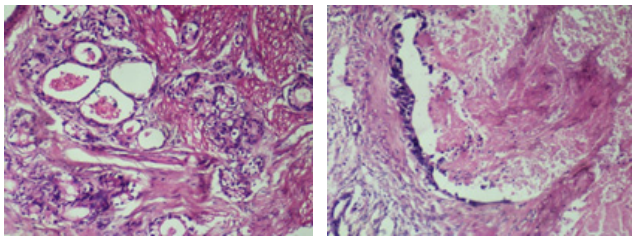

**Figure 2. Dysplasia in RAS - invasive GBC (H&E stain, X100)**

recognized. A key differentiating feature from adenomyosis is that muscular hypertrophy was not pronounced. Cytologic atypia sufficient for a diagnosis of adenocarcinoma was also evident. Therefore, careful examination of resected gallbladders is necessary; particularly in areas of focal mural thickening.[19] Carcinoma arising from RAS is small and has a relatively good prognosis.

Adenomyosis can also be confused with adenocarcinoma of gallbladder (Case C). Adenomyoma or adenomatous hyperplasia is the mural collection of cystically dilated glands. Unlike RAS in adenomyosis there is no evidence of injury, no other sinuses, no communication with surface mucosa and muscular process.[20] However adenomyosis with invaginations extending into the thick muscular layer of gallbladder wall mimics well-differentiated adenocarcinoma of the gallbladder (Fig. 3). Pathologists should be aware of the presence of glandular structures embedded in gallbladder wall. This condition does not simply suggest RAS or adenomyosis. Adenomyomas with diffuse dysplastic changes with papillary configuration can be Mural intraductal papillary neoplasm.[20] The precise evaluation of the appearance of the whole lesion may be useful in distinguishing these diseases.

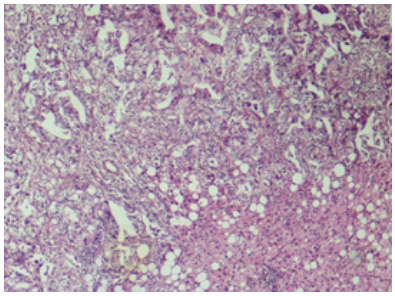

**Figure 3. Tumor cells invading upto muscular and serosal layer, may be confused with adenomyosis (H&E stain, X100)**

One of our cases was diagnosed as squamous cell carcinoma (SCC) in the gall bladder (Case D). Squamous cell carcinoma of gall bladder is of rare occurrence involving 1-4% of all cases. Large cholesterol gallstones, Clonorchis sinens is infection are associated with SCC of the gallbladder.[21] However on further work up of the patient cribriform pattern of tumor cells and with areas of comedonecrosis (initially reported as necrosis within the tumor nests) was seen. These features were the features of adenocarcinoma which in our case was mistaken for squamous cell carcinoma. Further Immunohistochemistry and molecular study of the sample was needed for confirmation which gave the diagnosis of adenocarcinoma.

Similarly another case of 50 years old female showed features of both adenocarcinoma and squamous cell carcinoma. Hence the diagnosis of adenosquamous carcinoma was made. Proper and detailed examination of the specimen is necessary for diagnosis.

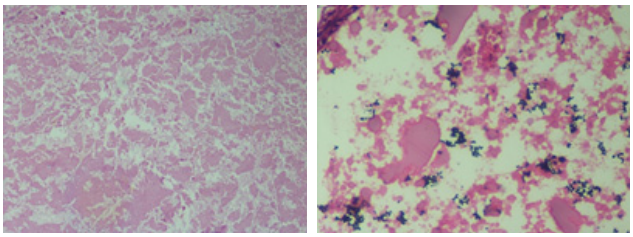

**Figure 4. Necrosis can mask underlying GBC. (H&E stain, X100)**

Another common problems faced by pathologist is that the pathological findings might overlap and may contribute to confusion, such as the presence of necrosis (Case E) (Fig. 4) or the presence of extracellular mucin. An acute cholecystitis with necrosis can be confused with a neoplastic process. One of our cases of 58 years old female (Case F) presented with features of acute cholecystitis which on further work up was found adenocarcinoma.

Similarly, tumor necrosis with minimal residual tumor may mimic acute gangrenous cholecystitis. Almost all cellular detail is lost in necrosis and most immunohistochemistry stains are inconclusive. The characteristics of the histologic changes in acute cholecystitis such as edema,vascular congestion, hemorrhage, fibrin deposition in the adventitia and adjacent muscle should be noted. Thorough histological sampling of the portion of gallbladder with or without necrosis to reveal diagnostic viable tumor is necessary.

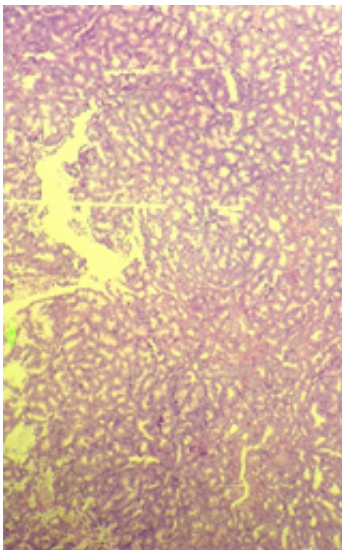

**Figure 5. Intracholecytic papillary tubular neoplasm (H & E; 100X)**

Another pitfall seen in our case is a mass sent separately from gall bladder with polypoidal features. The diagnosis of the 29 years old male (Case G) (Fig. 5) presented with features of Gall bladder polyp was Intracholecystic papillary neoplasm (ICPN). ICPN is defined as a grossly

visible, intraluminal growing mass-forming neoplasm and is histologically lined by epithelia presenting papillary/villous lesions as well as tubular structures with fine fibrovascular stalks and minimal intervening stroma.[22]

ICPN with associated invasive carcinoma is identified in approximately half of all resected ICPNs, particularly in lesions with a predominantly biliary morphology or extensive high-grade dysplasia.[23] However since the mass is sent separately in our case invasion could not be reported.

Two of our cases (Case H, I) illustrate the challenging aspect of gallbladder microanatomy. Surface dysplasia was identified on initial evaluation of case. Subsequently submitted deeper sections or regrossed sections also cannot demonstrate areas with dysplasia extended into deeply situated RAS. These features must be differentiated from reactive atypia and with invasion. Invasive tubules are relatively small, irregular and arranged haphazardly.[20]

Similarly Ducts of Luschka which are a developmental abnormality found within the gallbladder fossa may demonstrate reactive atypia that can be mistaken for invasive adenocarcinoma.[14] Histologically, Ducts of Luschka are composed of lobular aggregates of small ductules lined by bland, cuboidal-to-columnar biliary-type epithelium.[24]

Gall bladder tumor with features of adenocarcinoma can be a nuisance if it has to be differentiated from the metastatic adenocarcinoma. However metastatic spread to the gallbladder is extremely rare. Metastasis to the gallbladder is found in 2.2% to 5.8%. In those studies, gastric cancer was the most common site of primary origin.[15,25] Diagnostic confusion may arise because of mucosal colonization by metastatic colorectal carcinoma, i.e. growth along an intact basement membrane and colonization of the existing epithelium, thereby mimicking a primary tumor. IHC study and clinical history is necessary for diagnosis of tumor. In our study there was no case of metastasis to the Gallbladder.

Tumors contain more than one histological variants can also be found. For eg: mixed endocrine and exocrine carcinoma. Therefore, the differential diagnosis of a primary NEC of the gallbladder and one arising from metastasis is difficult.[5,26] Immunohistochemistry staining for cytokeratin and synaptophysin are helpful.[27]

There were few limitations in the study. This was a single center study with few limited samples. Secondly more experienced experts/pathologists could have been involved in the study for better interpretation.

## CONCLUSION

Carcinoma of the gallbladder is of poor prognosis. Mistakenly diagnosing gallbladder carcinoma with samples having features of deeply penetrating RAS or adenomyosis were common. Similarly confusing presence of mucin and necrosis were the common pitfalls in diagnosis of GBC. Careful attention to any evidence of mural thickening, thorough sampling, and close examination of deeply situated glandular structures that mimics RAS are critical for proper diagnosis of cases.

## REFERENCES


1. Stinton LM, Shaffer EA. Epidemiology of gallbladder disease: cholelithiasis and cancer. *Gut Liver*. 2012;6:172-87.
2. Anonymous: Cancer Facts and Figures. 2002 [http://www.cancer.org/downloads/STT/CancerFacts&Figures2002TM.pdf].
3. Tamrakar D, Paudel IS, Adhikary S, Rauniyar B, Pokharel PK. Risk factors for gallbladder cancer in nepal-A case control study. *Asian Pac J Cancer Prev.* 2016 Jul 1;17(7):3447-53.
4. Albores-Saavedra J, Chable-Montero F, Angeles-Albores D, Schwartz A, Klimstra DS, Henson DE. Early gallbladder carcinoma: a clinicopathologic study of 13 cases of intramucosal carcinoma. *Am J Clin Pathol.* 2011;135(4):637-42.
5. Giang TH, Ngoc TT, Hassell LA. Carcinoma involving the gallbladder: a retrospective review of 23 cases - pitfalls in diagnosis of gallbladder carcinoma. *Diagn Pathol*. 2012 Jan 27;7:10. doi: 10.1186/1746-1596-7-10. PMID: 22284391; PMCID: PMC3285085.
6. Mondal SK, Bhattacharjee D, Mandal PK, Biswas S. Histopathological study of gallbladder carcinoma and its mimics with role of carcinoembryonic antigen immunomarker in resolving diagnostic difficulties. *Indian J Med Paediatr Oncol*. 2017 Oct-Dec;38(4):411-5. doi: 10.4103/ijmpo.ijmpo_230_15. PMID: 29333003; PMCID: PMC5759055.
7. Memon MA, Anwar S, Shiwani MH, Memon B. Gallbladder carcinoma: a retrospective analysis of twenty-two years experience of a single teaching hospital. *Int Semin Surg Oncol.* 2005 Mar 17;2(1):1-5. doi: 10.1186/1477-7800-2-6. PMID: 15774016; PMCID: PMC1079924.
8. Mishra R, Goda C, Arora M, Sood M, Siddiqui AA, Husain A, et al. Treatment of Gall Bladder Cancer: A Review. *Indo Glob J Pharm Sci*. 2012 Mar;2(1):54-62.
9. Ghimire P, Yogi N, Shrestha BB. Incidence of incidental carcinoma gall bladder in cases of routinecholecystectomy. *Kathmandu Univ Med J.* 2011 Apr-Jun;9(34):3-6.
10. Onoyama H, Yamamoto M, Takada M, Urakawa T, Ajiki T, Yamada I, et al. Diagnostic imaging of early gallbladder cancer: retrospective study of 53 cases. *World J Surg.* 1999;23:708-12. doi: 10.1007/pl00012373
11. Oikarinen H, Paivansalo M, Lahde S, Tikkakoski T, Suramo I. Radiological findings in cases of gallbladder carcinoma. *Eur J Radiol.* 1993;17:179-83.
12. Archer A, Horton K. Radiologic evaluation and treatment of gallbladder and biliary tree carcinoma. *Cancer Treat Res*. 1994;69:157-83.
13. Kim JH, Ryu SM, Choi CS, Yoon HK, Choi YK. Expression of muc gene proteins in cholecystitis, adenoma and adenocarcinoma of the gallbladder. *J Korean Surg Soc*. 2005;69(2):139-45.
14. Rajab R, Meara N, Chang F. Florid ducts of Luschka mimicking a well differentiated adenocarcinoma of the gallbladder. *The Int J Pathology*. 2007;6:360-5.
15. de Bitter TJJ, Trapman DM, Simmer F, Hugen N, de Savornin Lohman EAJ, de Reuver PR, et al. Metastasis in the gallbladder: does literature reflect reality? *Virchows Arch*. 2022 Jun;480(6):1201-9. doi: 10.1007/s00428-022-03314-7. Epub 2022 Mar 31. PMID: 35357569; PMCID: PMC9184415.

16. Albores-Saavedra J, Shukla D, Carrick K, Henson DE. In situ and invasive adenocarcinomas of the gallbladder extending into or arising from Rokitansky-Aschoff sinuses: a clinicopathologic study of 49 cases. *Am J Surg Pathol.* 2004;28(5):621-8.

17. Saul SH: Gallbladder and extrahepatic biliary tree. Sternberg's Diagnostic Surgical Pathology. 2009, LWW, Chapter 35: 1205-1227.

18. Pandey M, Pathak AK, Gautam A, Aryya NC, Shukla VK. Carcinoma of the gallbladder. A retrospective review of 99 cases. *Dig Dis Sci.* 2001;46(6):1145-51.

19. Terada T. Gallbladder adenocarcinoma arising in Rokitansky-Aschoff sinus. Case report. *Pathol Int.* 2008, 58:806-9.

20. Sternberg SS, Mills SE, Carter D, editors. Sternberg's diagnostic surgical pathology. Lippincott Williams & Wilkins; 2004.

21. Willson CM, Barsoum N, Khan MTA, Rushton J. Primary Squamous Cell Carcinoma of Gallbladder With Hepatic Invasion. *Cureus*. 2023 Mar 5;15(3):35795. doi: 10.7759/cureus.35795. PMID: 37025738; PMCID: PMC10072881.

22. Nakanuma Y, Nomura Y, Watanabe H, Terada T, Sato Y, Kakuda Y, et al. Pathological characterization of intracholecystic papillary neoplasm: A recently proposed preinvasive neoplasm of gallbladder. *Ann Diagn Pathol*. 2021 Jun;52:151723. doi: 10.1016/j.anndiagpath.2021.151723. Epub 2021 Mar 3. PMID: 33725666.

23. Watanabe Y, Mochidome N, Nakayama H, Gotoh Y, Setoguchi T, Sunami S, et al. Intracholecystic papillary neoplasm associated with invasive carcinoma of the remnant gallbladder after subtotal cholecystectomy: a case report. *Surg Case Rep*. 2022 Feb 21;8(1):31. doi: 10.1186/s40792-022-01388-8. PMID: 35187597; PMCID: PMC8859015.

24. Singhi AD, Adsay NV, Swierczynski SL, Torbenson M, Anders RA, Hruban RH, et al. Hyperplastic Luschka ducts: a mimic of adenocarcinoma in the gallbladder fossa. *Am J Surg Pathol*. 2011 Jun;35(6):883-90. doi: 10.1097/PAS.0b013e3182196471. PMID: 21566517; PMCID: PMC3568524.

25. Disibio G, French SW. Metastatic patterns of cancers: results from a large autopsy study. *Arch Pathol Lab Med*. 2008;132(6):931-9. doi: 10.5858/2008-132-931-MPOCRF.

26. Niu C, Wang S, Guan Q, Ren X, Ji B, Liu Y. Neuroendocrine tumors of the gallbladder. *Oncol Lett*. 2020 May;19(5):3381-8. doi: 10.3892/ol.2020.11461. Epub 2020 Mar 12. PMID: 32269610; PMCID: PMC7115111.

27. Hussain I, Sarvepalli D, Zafar H, Jehanzeb S, Ullah W. Neuroendocrine tumor: a rare, aggressive tumor of the gallbladder. *Cureus.* 2019 Sep 5;11(9):e5571